# SYNOPSIS

## (1) TITLE OF THE SYNOPSIS

### "*0*-NUCLEOPHILICITY OF HYDROXAMIC ACID: ESTEROLYTIC CLEAVAGE OF SOME SIMULANTS OF CHEMICAL WARFARE AGENTS AND ORGANOPHOSPHORUS PESTICIDES"

## (2) INTRODUCTION

Numerous organophosphorus compounds are widely used in the agriculture all over the world as pesticides and insecticides. Organophosphorus nerve agents used as a chemical warfare weapons (**Chart-1**) are chemically related to organophosphates used as pesticides (**Chart-2**), but they are more toxic to mammals, particularly when absorbed through the skin[1-4]. As a matter of fact, both of them exert their toxicity through nonreversible phosphorylation and inactivation of esterase in the central nerve system[5-7]. The most important enzyme inhibited by organophosphorus compounds is the acetylcholenesterase (AChE), which is responsible for regulating the concentration of the neurotransmitter acetylcholine at cholinergic synapses. Inactivation of AChE results in the buildup of neurotransmitter, acetylcholine (ACh) and produce serious clinical complications including respiratory disorders and fibrillation and leads ultimately to death[8-12].

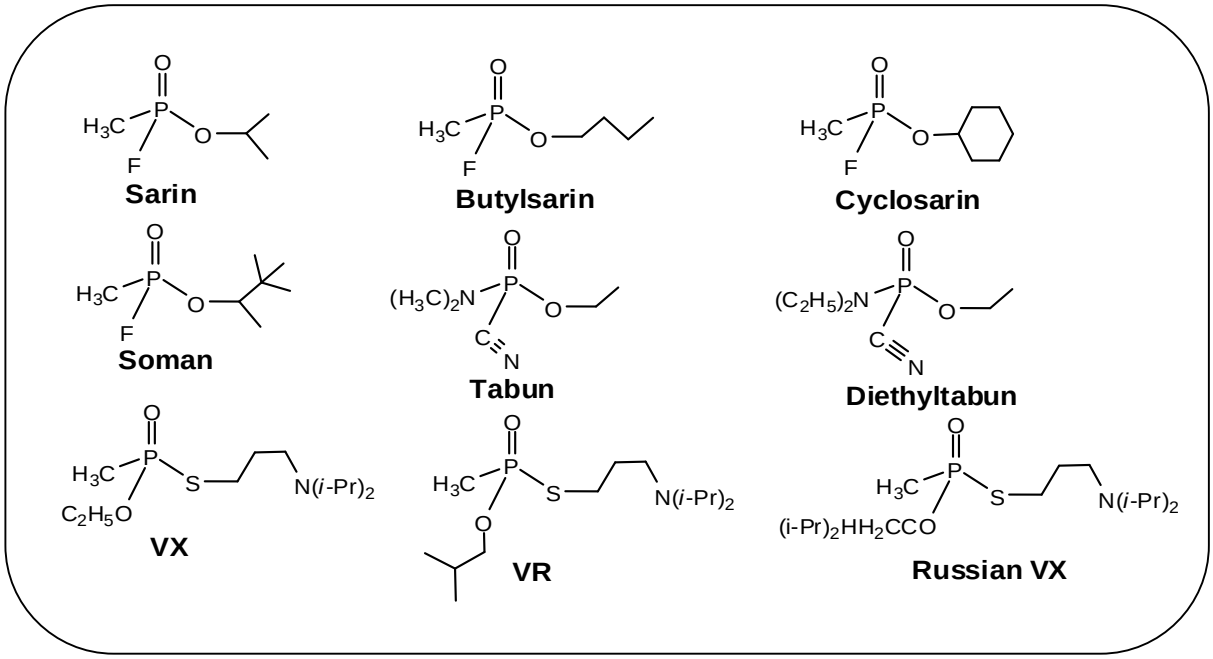



**Chart-1**: Structure of some Chemical Warfare Agents

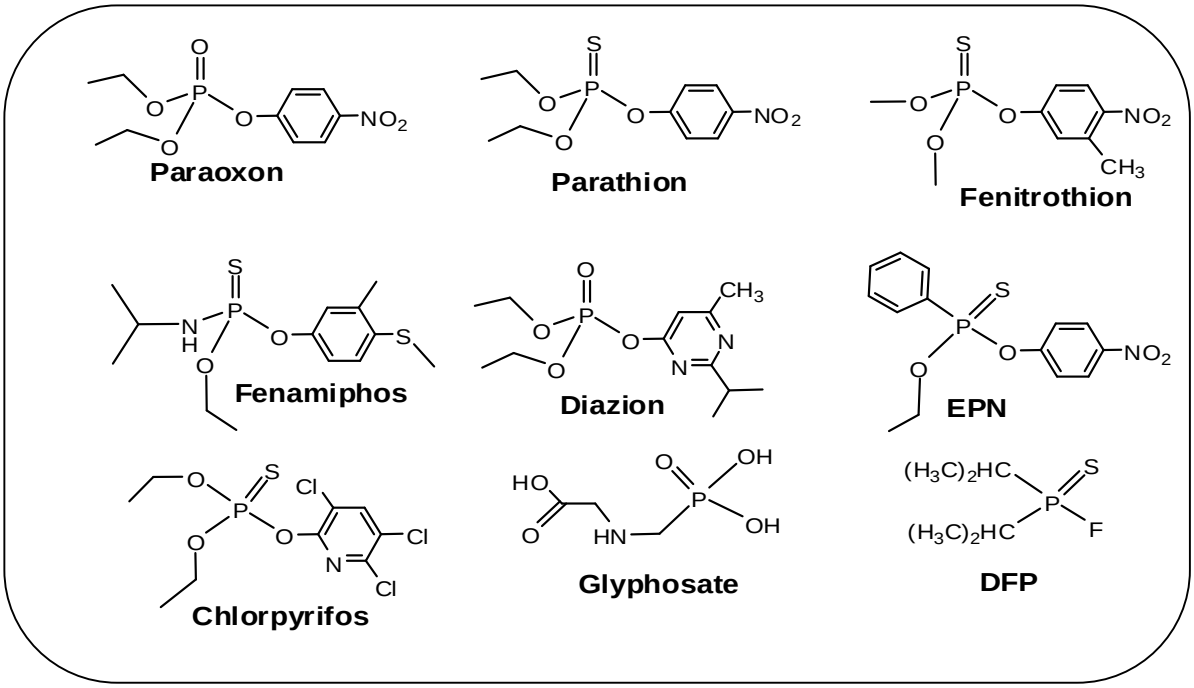



**Chart-2**: Structures of Organophosphorus Pesticides

The use of organophosphorus pesticides in agriculture or possible misuse of such agents by terrorist has clearly established an urgent need for rapid, sensitive, and reliable methods for detection and decomposition on this compounds[13-14]. The wide-spread organophosphorus compounds (OP) as pesticides and availability of highly toxic OP-type chemical warfare agents (nerve agents) underline the necessity or an effective medical treatment. Acute toxicity is primarily caused by inhibition of acetylcholenesterase (AChE). Reactivators (oximes) of inhibited AChE are a mainstay of treatment, pralidoxime, HI 6 and HLo 7(**Chart-3**) are considered to be rather effective against various nerve agents[15]. The reactivation kinetics of the said oximate-based reactivators is based on the nucleophilic cleavage of aged organophosphate-enzyme complex. Hydroxamic acids are functional analogues of the oximes, but, surprisingly no reactivators so far reported from hydroxamic acid family. Therefore, it is proposed to study the nucleophilic reactivity of hydroxamate ion for the cleavage organophosphorus compounds.

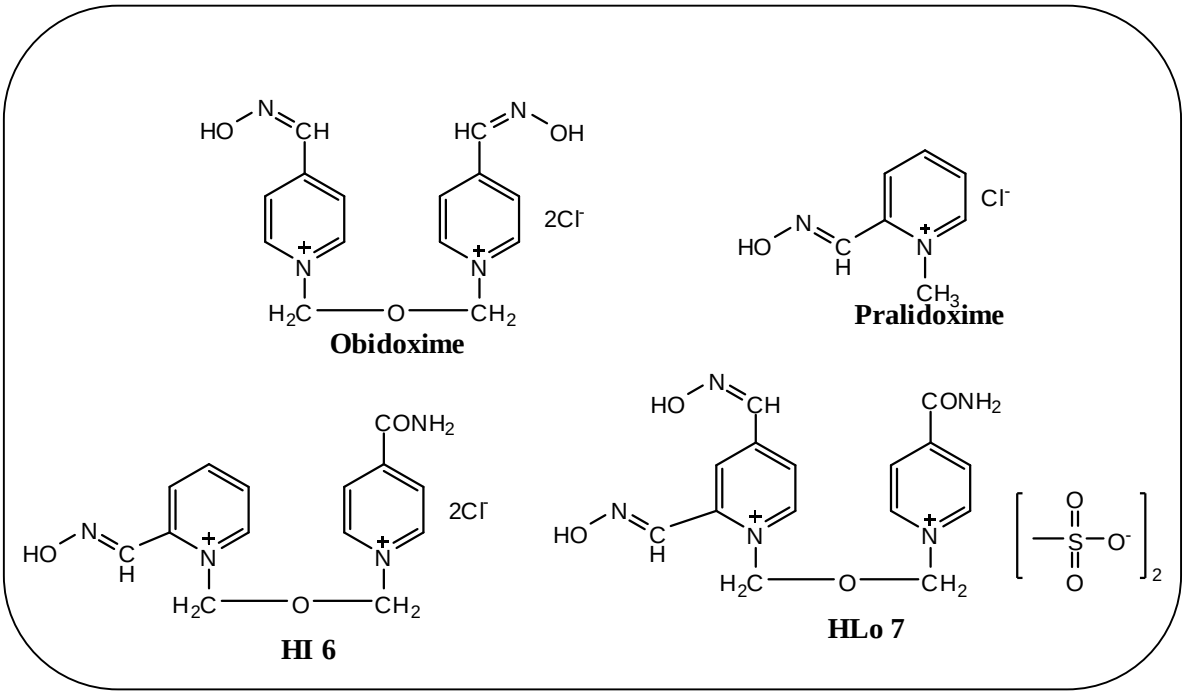



**Chart-3**: Oxime Based Reactivators

## (3) REVIEW OF THE EARLIER WORK

Estererolytic reaction of the carboxylate, phosphate, thiophosphate and sulfonate has recently received intensive attention due to the importance in chemical and biological processes. The phosphotriester-based paraoxon and parathion are used as pesticides, are most frequently responsible for the poisoning of agriculture field workers. Considerable research efforts have been focused on the nucleophilic reagents such as monoperoxyphthalate, 4-*N*, *N*-dialkylaminopyridines and metallomicelles. Recently, Bhattacharya *et al.*[16-18] reported the ester cleavage properties and significant turn over catalysis of synthetic hydroxybenzotriazoles[16-17] and 5-alkyl-1H-tetrazoles[18]. Dubey *et al.*[19] investigated micellar effects on hypochlorite catalyzed decontamination of toxic phosphorus esters. Most significant effort have been given by their research group on the detection of hydrolytic products of the chemical warfare agents and organophosphorus pesticides, which are the signature of the exposure of these toxic compounds[20-22]. They developed a simple and economical neutralization method against highly toxic chemical warfare agent's viz. sulfur mustard (SM), sesquimustared, and their homologues/analogues. The method involves treatment of chemical warfare agents with sodium in inert solvents. This distraction method of sulfur mustards release innocuous products via desulfurization reaction. The products were characterized by GC-MS technique. The method is suitable in particular, for bulk destruction of heel of mustered stockpiles. In recent year much attention has been focused on the remarkable acceleration of

phosphodiester and triester hydrolysis using metal ions. Shined *et al.*[23] reported the metal ion mediated hydrolytic decomposition of methyl parathion and cynofenophos in acidic media.

The role of metal ion in catalysis of phosphoryl transfer reactions has been considered in numerous reviews[24-26]. Recently, Brown *et al.*[27] reported a method for the decomposition of paraoxon via a methanolysis reaction catalyzed La (Otf). That metal ion proved to be remarkably effective in accelerating the methanolysis of inactivated and activated carboxylic esters[28], activated amide such as $\beta$-lactum[28a] and certain phosphate esters such as diphenyl phosphate, methyl *p*-nitrophenyl phosphate bis(*p*-nitrophenyl) phosphate[28b] and 2-hydroxypropyl *p*-nitrophenyl phosphate[28c]. In the case of neutral substrates such as carboxylate esters catalyzed by $\beta$-lactum and paraoxan, the dominantly active form of the catalyst is a $La^{3+}$ dimmer with two methoxides. Quite recently Pt and Pd metallocycles were shown to be efficacious for thiophosphate pesticides hydrolysis[29]. Hengge *et al.*[30] reported macrocyclic dinuclear ZnL complex and studied carboxyl ester hydrolysis of 4-nitrophenyl acetate catalyzed by this Zn (II) complex.

## (4) OBJECTIVES OF THE STUDY

The specific objectives of the present investigation are:-

(i) Synthesis and characterization of hydroxamic acids containing multiple reactive sites.

(ii) To design a catalytic system capable of degrading organophosphorus compound in catalytic manner i.e. turn over catalysis.

(iii) To investigate the effective multifunctional catalyst for cleavage of stimulants of chemical warfare agents and organophosphorus esters.

(iv) To study the stability of the hydrolysis against the hydrolysis in acidic and alkaline conditions.

(v) To study the nucleophilic reactivity of hydroxamic acid with phosphate esters in micelle/microemulsion.

## (5) NOTEWORTHY CONTRIBUTIONS IN THE FIELD

Hydroxamic acids are group of weak organic acid. There are crucial role of hydroxamic acid in various field such as medicine and biology, for example as siderophores for iron(III), as a potent and selective inhibitors of enzymes such as peroxidases, ureases and matrix metalloprotiens and as hypotensive, anti-cancer, anti-tuberculosis and antifungal agents. Hydroxamate ions are $\alpha$-effect

nucleophiles and are strong deacylating and dephosphorylating agents[31]. Satnami, *et al.*[32] recently reported bifunctional nucleophilic attack of salicylhydroxamate ion at C=O and P=O centers in cationic micellar media. The nucleophilic catalysis by acetohydroxamate, benzohydroxamate and salicylhydroxamate ions has been extensively investigated[33-34]. Present synopsis aims to investigate the catalytic hydrolysis of organophosphorus compounds which are used as stimulants of chemical warfare agents using bifunctional/long chain hydroxamic acids in micellar/microemulsion media.

# (6) PROPOSED METHODOLOGY DURING THE TENURE OF THE RESEARCH WORK

The entire experimental work will be done in the following steps:-

## I. Synthesis and Characterization of Hydroxamic Acid.

All the hydroxamic acids will be synthesized and characterized by IR, $^{1}$HNMR, $^{13}$CNMR and Mass spectral an analysis.

Pyridinhydroxamic acids such as 2-, 3- and 4-pyridinhydroxamic acid will be prepared by hydroxylamine hydrochloride and their corresponding esters in alcohol in the presence of sodium hydroxide. The synthesis of imidazole-4-carbohydroxamic acid involves the two step synthesis. In the first step, imidazole-carboxylic acid will converted to the corresponding acid chloride by $PCl_5$ according to the procedure of Wegner and Weidenhagen.

*N*-Methylsalycilhydroxamic acid will be prepared in chloroform in the presence of triethylamine from salicyloyl chloride and *N*-Methylhydroxylamine hydrochloride. The crude product will be purified in the form of copper chalets and then recristallized from water. *N*-Methylphalohydroxamic acid will be synthesized by the reaction of phthalic anhydride and *N*-Methylhydroxilamine hydrochloride in the presence of triethylamine. Long chain hydroxamic acid group i.e. *N*-hydroxy octanamide, *N*-hydroxy-decanamide , *N*-hydroxy-palmitamide and *N*-hydroxy stereamide will be prepared in ethanol in the presence of sodium hydroxide from corresponding esters.

## II. Determination of p$K_a$ of the Hydroxamic Acid.

The p*K*a values of all the synthesized hydroxamic acids and some oximes will be determined by pH-metric, and spectrophotometric titration and will be calculated from Albert and sergent method as

per eq. (1). The apparent p$K_a$ value will be determined by kinetic measurement also. Therefore the p$K_a$ value of all the long chain hydroxamic acid will be determined under the micellar condition

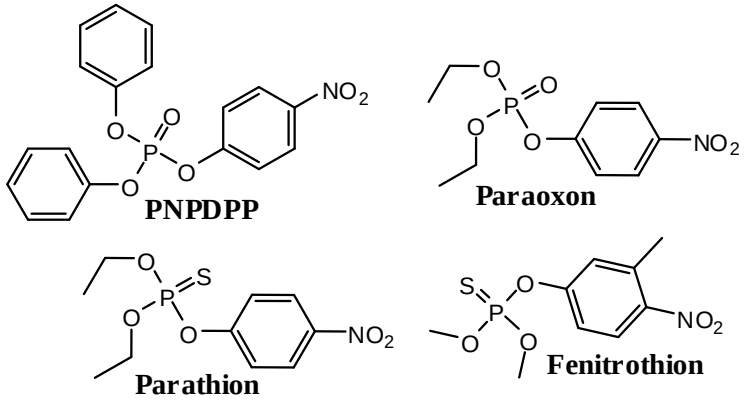
$$pK_a = pH - \log \frac{Abs_{\Psi} - Abs_{HA}}{Abs_{A^-} - Abs_{\Psi}} \quad \text{------ (1)}$$

Where

$Abs_{\psi}$ = is the absorbance of the partially ionized (at observed pH) from the oxime
$Ab_{HA}$ = is the absorbance of the unionized form of the oxime and
$Abs_{A^-}$ = is the absorbance of the completely ionized form of the oxime.

## III. Nucleophilic cleavage of Carboxylate and phosphate esters by Hydroxamate ions.

Pseudo-first-order rate constants for the reaction of *p*-nitrophenyl benzoate (**PNPB**), tris (3-nitrophenyl) phosphate (**TRIS**) or *p*-nitrophenyl diphenyl phosphate (**PNPDPP**) Paraoxon, Parathion and Fenitrothion with hydroxamate ion will be determined at 27$^0$C with the nucleophiles in large excess over the substrate. pH-dependent pseudo-first-order rate constants will be determined in the 6-12 pH range.



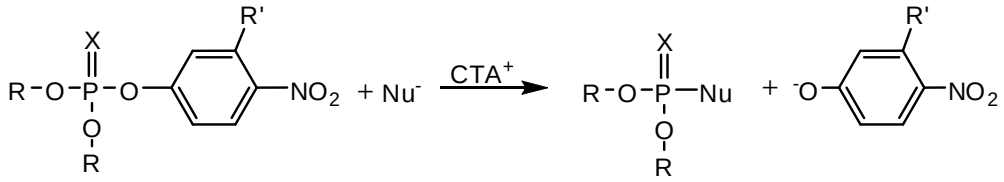

All of the reactions will be followed at 27$^o$C with a UV-visible spectrophotometer. The rate of nucleophilic reaction with esters will be determined by following the increases in absorption of nitrophenoxide anion (400nm). All of the kinetic experiments will be performed at an ionic strength of 0.1M (KCl). Phosphate and borate buffer will be employed to obtain desired pH of the reaction medium. All reaction will be conducted under pseudo-first-order conditions. For all kinetic runs, absorption/time result fit very well to the first-order rate equation (2).

$$\ln(A_\infty - A_t) = \ln(A_\infty - A_0) - kt \quad = \quad \text{.....(2)}$$

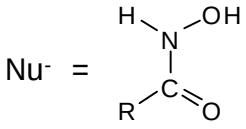
The pseudo-first-order rate constants can be determined by least squares fits each experiment will be repeated at least twice and the observed rate constant will be obtained to be reproducible within a precision of about 3% or better.

## IV. Esterolytic Cleavage of Phosphate Esters by Co-micellized long–chain Hydroxamate ions.

Reactivaties of hydroxamate ions are increased by incorporation in functional polymers or by comicellization with inert surfactants in water. As expected on the basis of pseudo phase treatment of miceller rate effects, high local concentration of hydroxamate ions in the interfacial miceller region are largely responsible for these rate increases. With the aim that miceller incorporation of long- chain hydroxamic acids will accelerate the hydrolytic cleavage of carboxylate and phosphate esters, the nucleophilic hydrolysis of *p*-Nitrophenyl diphenyl phosphate and organophosphorus pesticides (Paroxon, Parathion, Fenitrothion) will be studied in cetyltrimethylammonium based miceller media (**Scheme**-**1**)




R = $CH_3$, R' = $CH_3$, X = S, Fenitrothion
R = $C_2H_5$, R' = H, X = O, Paraoxon
R = $C_2H_5$, R' = H, X = S, Parathion
R = $C_6H_5$, R' = H, X = O, PNPDPP




R = $C_7H_{15}$ (Caprylohydroxamic acid), R = $C_9H_{19}$ (Decanohydroxamic acid), $R_{11}H_{23}$ (Lauryl hydroxamic acid), R = $C_{13}H_{27}$ (Myristyl hydroxamic acid); R = $C_{15}H_{31}$ (Palmityl hydroxamic acid), R = $C_{17}H_{35}$ (Stearohydroxamic acid)

**Scheme: 1:** Nucleophilic Reaction of Phosphate Ester and long-chain Hydroxamate ions.

The detailed investigation will be carried out under following step:

### (A) Micelle formation and Fractional ionization:

The intrinsic reactivity of amphiphilic or liphophilic reagent depends upon the extent of the micellar incorporation, which can alter the micellar morphology and critical micelle concentration, *cmc,* of the cationic micelles. Critical micelle concentration, *cmc* and fractional micellar ionization, $\alpha$, for the micelles of $CTA^+$ in the presence of buffer and hydroxamic acid (buffer at pH- 9.2) will be estimated from plots of conductance against surfactant. Break in these plots give *cmc* values. The fractional ionization constant will be calculated from the ratio of the slope of post micellar and pre-micellar region.

### (B) Intrinsic Esterolytic Reactivity of Co-micellized long-chain hydroxamate ion.

Kinetic of the nucleophilic cleavage of carboxylate and phosphate ester will be investigated. The kinetic reaction will be monitored spectrophotometrically, followed by measuring the time-based libration of nitrophenoxide ions. Rate effects on bimolecular reaction in association colloids are rationalized by the pseudo phase model, in which the aggregates and bulky solvent typically water, are regards as distinct reaction regions. The micellar catalyzed kinetic rate data will be treated quantitatively, applying pseudo-phase model. The turnover behavior of the micellized hydroxamte ions will be investigated under the condition in which substrate is in large excess than hydroxamate ion concentration. On the basis of experimental results, the mechanistic pathway of the nucleophilc reaction will be discussed.

## V. Catalytic decomposition of phosphate esters by hydroxamate ions in cationic microemulssion.

Most of the target organophosphorus compounds (nerve agents, insecticides, and their stimulants) are sparingly soluble in water. Thus the utility of micellar surfactant system is fairly a consequence of their capacity to solublize these organic materials in aqueous media.

In this section, nucleophilic catalytic hydrolysis of BDNPP and TRIS will be investigated in different formulation of cationic microemulsions. The cationic microemulsion will be prepared from the different weight percentage of oil (Hydrocarbon), water and surfactants dependent upon the excess amount of either oil or water, or the comparable composition of oil in water, (oil dispersed in water) water in oil (water dispersed in oil) and bicontinuous phase will be prepared. The kinetic

experimental will be performed to investigate the effectiveness of all the microemulsion system for hydrolytic reaction of phosphate esters. The most effective nucleophile will be utilized for catalytic reaction. Most emphasis will be given on the utility of the long-chain hydroxamate ions. Since long-chain hydroxamic acids are hydrophobic in nature; they could be regards as oil phase. pH-dependent rate constant, nucleophile dependent rate constant will be determined under the comparable micellar condition.

## VI. Comparative Nucleophilic Reactivity of Oximes and Hydroxamic acid.

It is interesting to note that hydroxylamine is parent compound for the three typical classes of the oxygen containing α-nucleophile: amidoximes, oximate, and hydroxamic acid. Surprisingly, large numbers of research have been done on oxime-based nucleophiles. The α-nucleophilicity of oximate ions and oximate-functionalized surfactants has been well documented.

This section aims to study the nucleophilic reactivity of oximate and hydroxamates of salicylic acid, phthalic acid, and pyridine-2-carboxylic acid (**Chart-4**) against the PNPB and BDNPP in cationic surfactants with various head and tails groups.

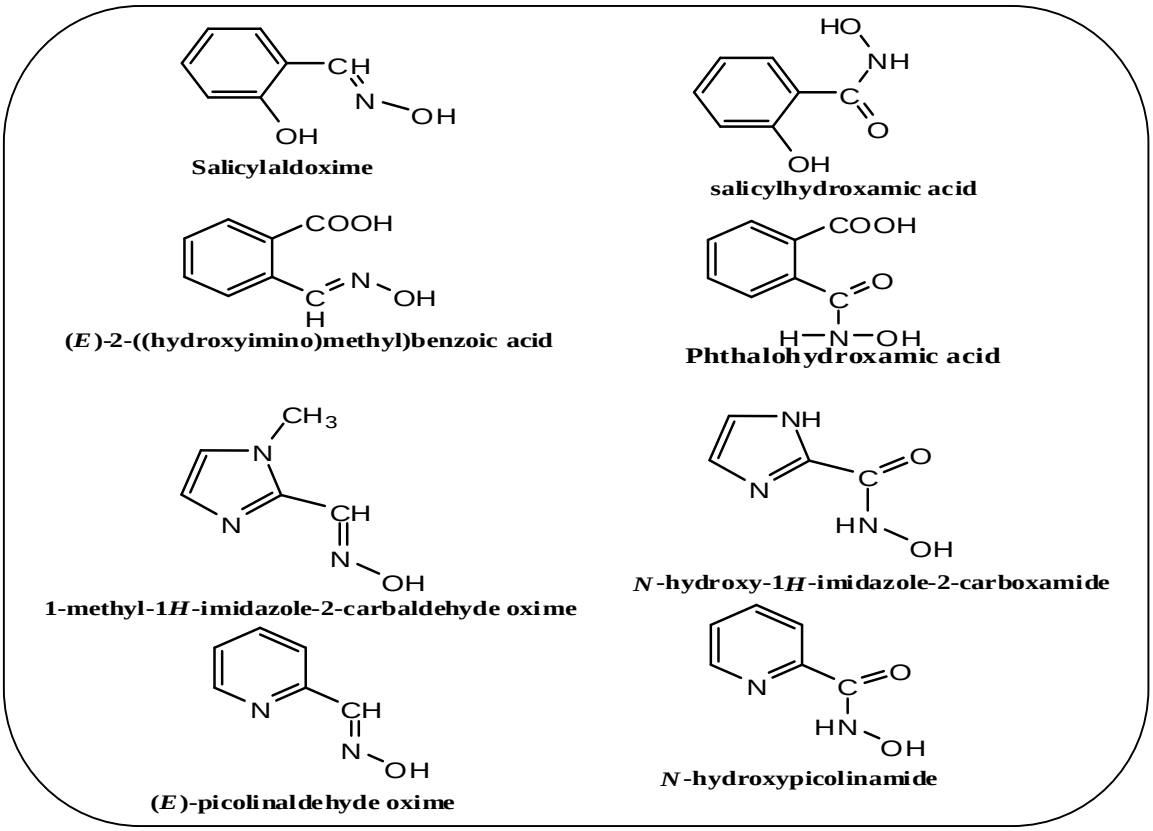



**Chart-4:** Structure of Oxime and Hydroxamic acid

## (7) EXPECED OUTCOME OF THE PROPOSED WORK

Given the practical importance of the decontamination and demilitarization of organophosphorus toxins, the search for efficient nucleophilic systems has been area of extensive research. Over the years, chemists have designed many nucleophiles, incorporating various modifications of the parent compounds, with the goal of obtaining more powerful reagents for the chemical degradation of organophosphorus substrates. For example, the parent oximate nucleophiles were modified to oximate-functionalized surfactants. In the similar manner, iodosylcorboxylate, iodosylbenzoate, iodosylnaphthoate and many iodosylcorboxylate- based functionalized surfactants were investigated, which displayed outstanding phosphorylating reactivity towards phosphate esters.

The catalytic activity of the hydroxamate ion based nucleophiles is in the level of parent compounds. This proposal is aimed to investigate the modification on parent hydroxamate based nucleophiles to enhance the efficiency. Further it is promising to developed hydroxamate-functionalized surfactants as potent esterolytic agents. After systematic investigating the problems following question may be answered:

i. How the hydroxamate ions could serve as good catalyst for hydrolysis of organophosphorus toxin?
ii. Do hydroxamate ions show “Turn-over” catalysis?
iii. What are the factors governing the nucleophilicity of hydroxamate ions?
iv. Which system (Micelle/microemulsion) provides better microenvironment for catalytic reactions?

The organized study will open the new insight on the development of hydroxamate-based catalyst for the hydrolysis of organophosphorus compounds.

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